Early-Season Plant Nitrate Test for Leaf Yield and Nitrate Concentration of Air-Cured Burley Tobacco

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ABSTRACT

Profitable yield and satisfactory leaf quality of burley tobacco (*Nicotiana tabacum* L.) require proper management of N fertilizer. The level of tissue NO_3^- in tobacco may be a suitable diagnostic test of crop N sufficiency that could be used for N management decisions. This study determined the suitability of early-season tissue NO_3^- as a predictor of air-cured leaf yield and NO_3^- concentration. Burley tobacco was grown in 1991 and 1992 on a well-drained Maury silt loam (fine, mixed, mesic, Typic Paleudalf) and a moderately well-drained Captina silt loam (fine, silty, siliceous, mesic Typic Fragiudult) broadcast fertilized just before transplanting with 0 to 392 kg N ha^{-1}. Tissue NO_3^- levels of plants sampled from 3 to 5 wk after transplanting and air-cured leaf yields increased with increasing amounts of fertilizer N. Responses differed depending on location and year. When the results were expressed as the percentage of the maximum within year and location, the relationship of leaf yield to early-season NO_3^- was described by a single linear equation ($y = 51.0 + 0.448x$; $r^2 = 0.808$, $P \leq 0.001$). However, to use this equation, it would be necessary to include in each tobacco field evaluated a strip of fertilizer N producing near maximum yield. The NO_3^- concentration of burley tobacco between 3 and 5 wk after transplanting was suitable for predicting the NO_3^- concentration of air-cured leaf lamina from the top, middle, and bottom stalk positions, and appeared to be insensitive to year and location effects. Use of plant NO_3^- concentration, as an early-season diagnostic test to predict N sufficiency and NO_3^- concentration of cured leaf lamina, appeared to offer promise for better N management of burley tobacco. Such a test during the interval between 3 and 5 wk after transplanting fits well into the normal cultivation practices. However, further research would be needed to calibrate the amount of additional N required to correct N deficits by banding N.

B URLEY TOBACCO is a high-value cash crop that receives large amounts of fertilizer to assure profitable yields. Inadequate N fertilization leads to decreased yields and market value (Miner and Sims, 1983). However, excessive use of N is costly, produces air-cured leaves with undesirable high NO_3^- (Brunneman and Hoffmann, 1982; Tso et al., 1975), and has the potential to degrade the quality of soil and water resources. For example, when N fertilizer practices recommended by the Kentucky Agricultural Experiment Station were followed for burley tobacco grown on moderately to well–drained soils (Anonymous, 1998), substantial NO_3^- was found in the soil profile (MacKown et al., 1999), a portion of which is probably leached below the root zone

of winter cereal cover crops. In addition, Mn toxicity induced by soil acidity is often observed in burley tobacco fields heavily fertilized with N (Hiatt and Ragland, 1963; Miner and Sims, 1983; Sims and Atkinson, 1973). Improved N management may overcome these negative effects.

Various early-season plant and soil diagnostic tests to better manage the N needs of other crops have been evaluated. Depending on the crop, some of these tests may be useful. Early-season plant tests for N sufficiency have included assessments of leaf chlorophyll, tissue total N, and tissue NO_3^- levels. Total N concentration of burley tobacco leaves sampled between 3 and 5 wk after transplanting appeared to be a better index of leaf yield than extracted chlorophyll or chlorophyll meter readings (MacKown and Sutton, 1998). However, use of these traits to predict corrective N fertilizer needs would require reference plots fertilized with a nonlimiting amount of N to account for the environmental effects and plant developmental variability. Early-season tissue NO_3^- as a diagnostic test of N sufficiency has not been evaluated in tobacco but has given promising results for several other crops (e.g., Constable et al., 1991; Knowles et al., 1991; Roth et al., 1989; Swiader et al., 1988; Westcott et al., 1991).

Our objective was to evaluate early-season plant NO_3^- concentrations within the first 5 wk after transplanting to determine if tissue NO_3^- tests could be used as an index of N sufficiency for predicting cured leaf yield and NO_3^- concentration in burley tobacco. Early-season measurements were restricted to the interval between 3 and 5 wk after transplanting, because this is a period when fertilizer could be applied easily during a normal cultivation of the crop and a rapid diagnostic plant test could be used to correct N deficiencies.

MATERIALS AND METHODS

Experiments were conducted in 1991 and 1992 on two University of Kentucky research farms near Lexington, KY. Details of the experimental sites and the previous cropping and cultural practices were described before (MacKown et al., 1999). The plots at Spindletop Research Farm were located on a well-drained Maury silt loam and those at Eden Shale Research Farm were on a moderately well-drained Captina silt loam. Previous cropping practices for the sites typified those where burley tobacco is produced. Recommended burley tobacco cultural and management practices were followed (Nesmith et al., 1993), except a broad range of N treatments was used. Broadcast applications of NH_4NO_3 (0–448 kg N ha^{-1} in 56-kg increments) were applied by hand and lightly disked into the soil surface 1 or 2 d before transplanting. The N treatments were arranged in a randomized complete block design with six replications. Plots were 15.2 m long and five rows wide except in 1991 when they were six rows wide at Eden Shale farm. Transplants of 'TN86' burley tobacco were

grown in conventional soil beds in 1991 and a greenhouse float system in 1992. In late May, transplants were set into the field at ≈0.49 m apart within rows spaced ≈1.02 m apart. Conventional practices (Nesmith et al., 1993) were used to cut and harvest the tobacco from mid August to early September (87–107 d after transplanting, depending on location and year). The harvested tobacco was housed in tobacco barns for air curing.

Plant Sampling and Nitrate Analysis

Plants were collected from a subset of the N treatments (0, 56, 112, 168, 280, and 392 kg N ha^{-1}). The first early-season sample was taken ≈3 wk after transplanting, and then additional plant samples were collected weekly for the next 2 wk. At least three plants were selected from the interior rows of a plot; each plant collected was surrounded on all sides by at least one plant. Plants were cut at the soil surface, rinsed with water, and then dried to a constant weight in a forced-air oven at 60°C.

Air-cured leaf yields were based on 40 to 50 plants harvested from the interior rows of a previously unsampled area of each plot. Air-cured leaves from each plot were grouped into top, middle, and bottom stalk positions, weighed, and subsamples from each stalk position were dried to a constant weight in a forced-air oven at 60°C.

The entire aboveground portion (leaves + stalk) of early-season plants was used for NO_3^- analysis. Midribs were removed from air-cured leaves and NO_3^- measured in the remaining leaf lamina. Tissues were ground to pass a 1-mm screen and redried to constant weight at 60°C before weighing samples for NO_3^- extraction for 1 h with deionized water at 97°C. Extracted NO_3^- was measured colorimetrically following microbial dissimilatory reduction to NO_2^- by a manual adaptation of an automated method (Lowe and Gillespie, 1975) or by an automated flow injection analyzer with a Cd reduction column.

Statistical Analysis

Analysis of variance and regression procedures (JMP version 3.2.2, SAS Institute, 1994) were used to analyze the data. Farm locations and year were initially treated as fixed effects in the analysis of variance model used to evaluate the response of air-cured leaf yield and NO_3^- concentration. A multivariate-fitting platform with repeated measures in time was used to analyze early-season plant dry weight and NO_3^-. When the sphericity test used to check the appropriateness of an unadjusted univariate F test for the within-subject effects was significant, the Geisser-Greenhouse adjusted univariate test was used to evaluate the F statistic determined for the contrast response design.

RESULTS

Early-Season Plant Dry Weight and Nitrate

Dry weight accumulation by aboveground plant biomass increased up to nearly ninefold between 3 and 5 wk after transplanting (Fig. 1). The overall average dry weight of plants collected at Spindletop was slightly greater (17%) than that of Eden Shale, but within each year the response of early-season dry weight to the level of N applied was not significantly different among locations. In 1991, the response of early-season growth at 4
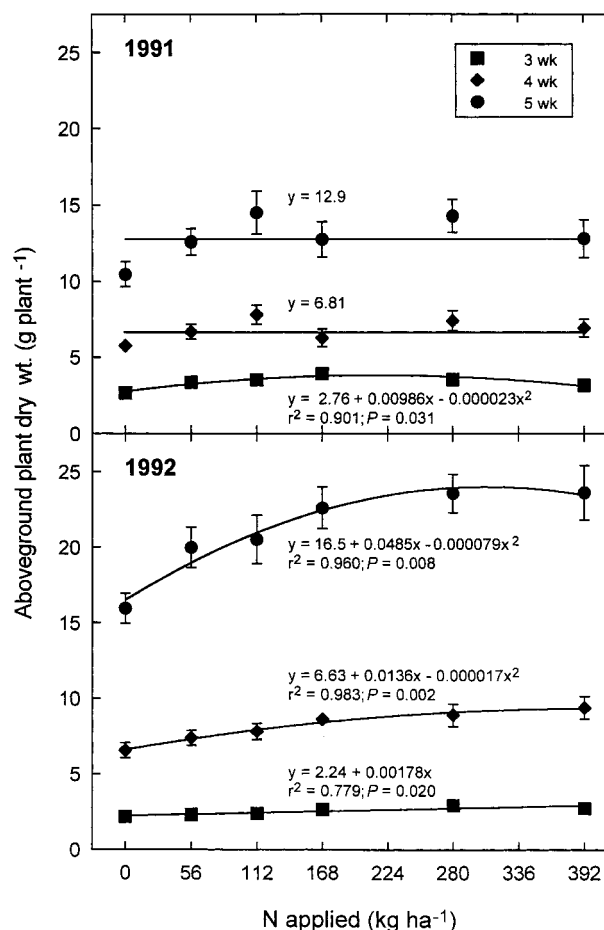

Fig. 1. Effect of N fertilizer applications on aboveground plant dry weight of burley tobacco at 3, 4, and 5 wk after transplanting. Data are means of Spindletop and Eden Shale farm locations and six replications of N treatments at each site in 1991 and 1992. Symbols with vertical bars represent ± 1 SE that exceed the size of the symbol.

and 5 wk after transplanting was unaffected by N fertilizer, whereas dry weight increased slightly in response to added N in 1992 (Fig. 1).

A significant sphericity test was found when the multivariate analysis of early-season tissue NO_3^- using sample date as a repeated measure was transformed into the univariate model. The adjusted univariate test was significant ($P < 0.001$) for the contrast design interaction of sample date with the between-subject model factor of N fertilizer × year × location. Concentrations of NO_3^- in aboveground tissue increased with increasing amounts of N fertilizer applied just before transplanting (Fig. 2). For almost all cases in 1991, tissue NO_3^- increased in a linear response to added N. In contrast, 1992 early-season tissue NO_3^- rates of increase diminished with increasing amounts of applied N. Overall, early-season tissue NO_3^- levels measured in 1992 exceeded those in 1991 by 55%.

Relationship of Air-Cured Leaf Yield and Lamina Nitrate to Early-Season Plant Nitrate

The fertilizer N treatments resulted in diminishing rates of increase in leaf yield with increasing amounts
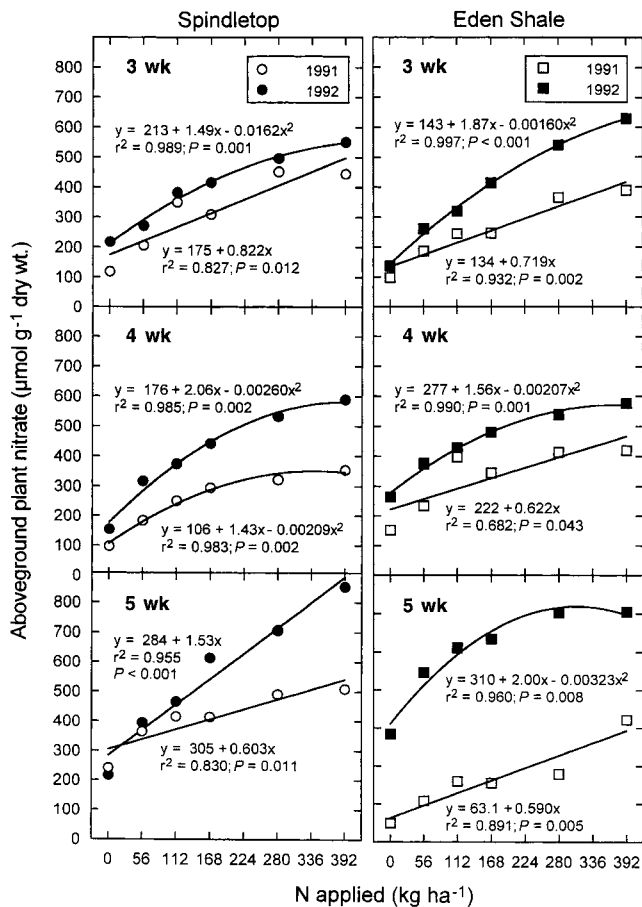
Fig. 2. Early-season (3, 4, and 5 wk post-transplant) responses of aboveground tissue NO_3^- concentrations of burley tobacco to broadcast N fertilizer applied just before transplanting at Spindletop and Eden Shale farms in 1991 and 1992.

Fig. 3. Changes in air-cured leaf yield (0.2 kg moisture kg^{-1} dry wt. of leaf) and the average aboveground tissue NO_3^- concentration of burley tobacco between 3 and 5 wk after transplanting in 1991 and 1992. Data points are N fertilizer treatment (0–392 kg ha^{-1}, increments of 56 kg) means of six replications.

of N fertilizer, and the range and maximum yields of air-cured leaf in 1991 were greater than those in 1992 (see Fig. 2 in MacKown et al., 1999). Within a year, the leaf yield response patterns to added N were not significantly different at the two locations, but overall the yields on the well-drained silt loam soil at Spindletop farm were greater than those on the moderately well-drained silt loam soil at Eden Shale farm. Based on similar positive linear relationships between yield of air-cured leaf and the concentration of NO_3^- in the total aboveground tissue of burley tobacco plants sampled at 3, 4, and 5 wk after transplanting, the data from the three early-season sample dates were combined (Fig. 3). The 1992 early-season tissue NO_3^- concentrations were greater and covered a broader range than in 1991, whereas the 1992 overall leaf yields were lower and covered a narrower range than in 1991. Within years, the slopes of the linear responses were significantly different for the two locations. When yields and 3- to 5-wk average NO_3^- concentrations were expressed as a percentage of the maximum for each year and location, the relationship between these two traits was represented by a single linear regression equation (Fig. 4).

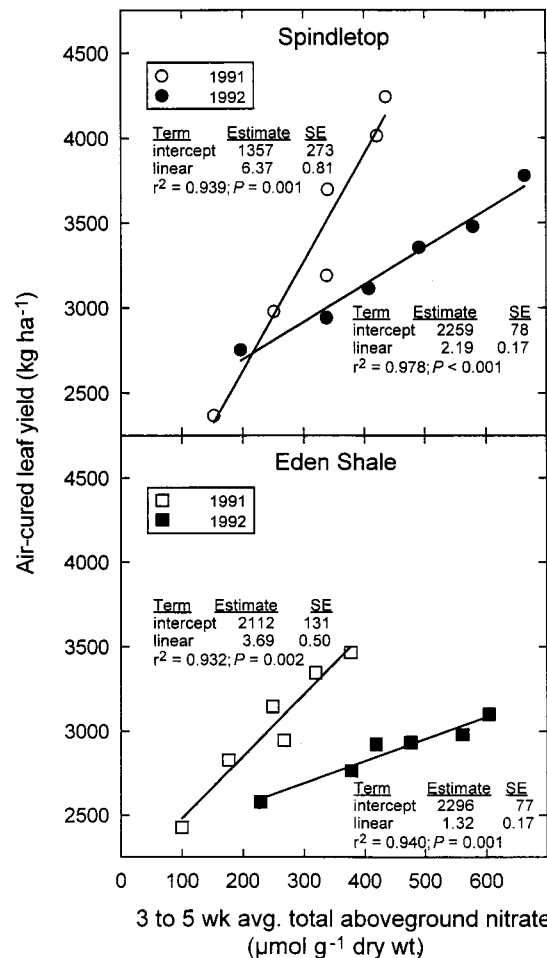Positive slope linear relationships between air-cured lamina NO_3^- and broadcast N fertilizer were observed except for 1991 at Eden Shale, where rates of increase in lamina NO_3^- concentrations diminished with increasing amounts of N fertilizer (see Fig. 5 in MacKown et al., 1999). The NO_3^- concentration of air-cured leaf lamina from bottom, middle, and top stalk positions increased with increasing NO_3^- concentrations of early-season aboveground tissue (Fig. 5). Relative changes in the NO_3^- concentration of air-cured leaves in response to early-season plant NO_3^- levels decreased from lower to upper leaves in the plant canopy. The relationships were concave quadratic for leaves from the bottom and middle stalk positions and linear for leaves from the top of the plant.

DISCUSSION

Although at 3 wk after transplanting the aboveground dry weight response to applied N in 1991 was only slightly different than in 1992, by 5 wk after transplanting the aboveground dry weight was considerably greater in 1992 and exhibited a concave quadratic response to applied N (Fig. 1). A cause for the difference in growth at 5 wk after transplanting could be related to differences in previous cropping practices, environ-
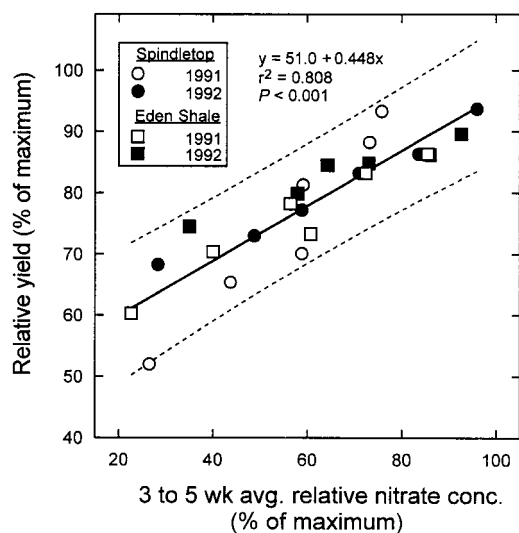
Fig. 4. Relationship between yield of air-cured leaves and the aboveground NO$_3^-$ concentrations of early-season burley tobacco plants sampled at 3, 4, and 5 wk after transplanting in 1991 and 1992. Data are expressed relative to the maximum value of each trait observed within each year and location. Dashed lines represent 95% confidence intervals that include both the variability of regression estimates and the variability of the observations making them suitable for prediction intervals.

Fig. 5. Relationships between NO$_3^-$ concentrations of air-cured burley tobacco lamina of leaves from top, middle, and bottom stalk positions and the average NO$_3^-$ concentrations of total aboveground tissues collected at 3, 4, and 5 wk after transplanting in 1991 and 1992. Dashed lines represent 95% confidence intervals that include both the variability of regression estimates and the variability of the observations making them suitable for prediction intervals.

mental conditions, and the method of transplant production. Immobilization of mineral N by a plowed cover crop and abundant early-season rainfall could decrease plant-available N. However, it is unlikely that the previous cropping practice in 1991 caused a greater immobilization of fertilizer N in 1991 than 1992. Cover crops of winter wheat (*Triticum aestivum* L.) were plowed in the spring, except at Eden Shale in 1991 when a 2-yr-old stand of red clover (*Trifolium pratense* L.) was plowed to prepare the site for tobacco. Environmental data for Spindletop farm (University of Kentucky Agricultural Weather Center, 1998) reveals that during the first 5 wk after transplanting, average daily air temperature in 1991 was ≈3°C warmer than normal, while in 1992 it was ≈3°C cooler than normal. Precipitation for this period was ≈53 mm greater than normal in 1991 and ≈26 mm greater than normal in 1992. In 1991, more abundant rainfall during the first 5 wk after transplanting could have decreased N availability. Lower early-season tissue NO$_3^-$ concentrations in 1991 than 1992 (Fig. 2) would be consistent with decreased available N and NO$_3^-$ uptake activity; however, levels of soil NO$_3^-$ in 1991 at 3 and 5 wk after transplanting were greater than or equal to those in 1992 (MacKown et al., 1999). Finally, this leaves the method of transplant production as a possible cause for greater early-season growth in 1992 than 1991. Transplants used in 1991 were obtained from conventional soil beds. Transplants used in 1992 may have been affected less initially because they were grown in a greenhouse float system that produces seedlings with soil-plugs and exposed roots. Suggs and Mohapata (1988) found that transplants obtained from greenhouse soil-plug–grown seedlings with intact roots generally grew faster than bare-root transplants pulled from seedling beds in the field. Despite the greater
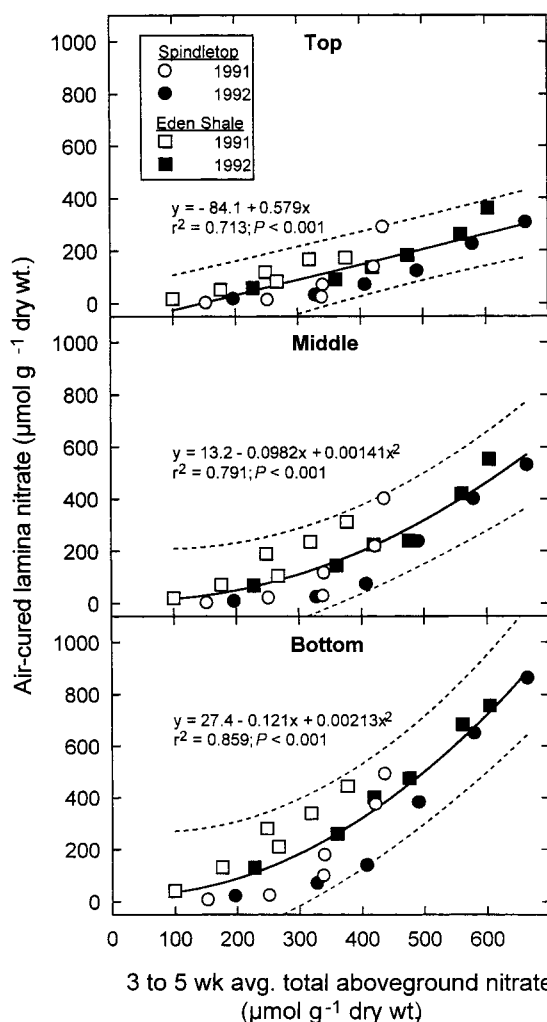
aboveground growth of tobacco in 1992 at 5 wk after transplanting, the average 1991 air-cured leaf yield maximums (and range) exceeded those in 1992 by ≈17% (MacKown et al., 1999). Consequently, growth during the first 5 wk after transplanting was not a good predictor of yield.

The response of early-season NO$_3^-$ concentration in the total aboveground plant tissue to applied N was consistent with that of stem and petiole responses observed with other crops (e.g., Constable et al., 1991; Knowles et al., 1991; Roth et al., 1989; Swiader et al., 1988; Westcott et al., 1991). Rather than use specific organs, as was done often for other crops, we sampled the entire aboveground portion of plants for two reasons. First, we believed that for producers or consultants the task of consistently selecting a particular plant part for diagnostic testing would prove more difficult than selecting representative plants from a field that would be combined for analysis. Second, we believed that the

tissue NO_3^- concentration of the entire aboveground portion of plants would integrate the widely different NO_3^- concentrations associated with stem, leaf midrib, and lamina tissue of young tobacco plants (MacKown and Jones, 1986). Chopping the bulked sample and taking a subsample for the diagnostic test may minimize the inconvenience of having a large composite sample for analysis. Because NO_3^- quick tests of expressed sap of plant tissues correlate highly with dry matter NO_3^- analysis of these tissues (e.g., Hartz et al., 1993; Kubota et al., 1997; Westcott et al., 1993), a subsample of bulked tobacco plants could be ground in a food blender to collect sap for a quick test of N sufficiency.

The yearly differences in early-season tissue NO_3^- concentrations were not due to biomass dilution effects, because 1992 aboveground plant dry weight and NO_3^- levels equaled or exceeded those of 1991 (Fig. 1 and 2). Differences between years in soil NO_3^- concentrations of the upper 30 cm of soil (MacKown et al., 1999) probably do not account for the yearly differences in tissue NO_3^- levels. Apparent NO_3^- uptake activity during the first 5 wk after transplanting was greater in 1992 than in 1991, which was consistent with the more vigorous early-season growth of plants in 1992 than 1991 (Fig. 1) and the similar or slightly lower levels of soil NO_3^- observed at 5 wk after transplanting (MacKown et al., 1999).

Location and year differences for the relationship of air-cured leaf yield to the average NO_3^- concentration of plants sampled between 3 and 5 wk after transplanting indicate that environmental and soil factors have strong effects on the relationship between these traits (Fig. 3). It is unlikely that a single equation will predict actual leaf yield of burley tobacco on the basis of an early-season diagnostic test of NO_3^- concentration. However, expressing each of these traits relative to the maximum observed within a location and year reveals that relative leaf yield is directly proportional to the relative NO_3^- concentration of early-season plants. Unfortunately, a producer wishing to use plant NO_3^- concentration as an early-season diagnostic test for N sufficiency and as a tool for fertilizer management would need to include within the tobacco field a N-fertilized reference strip that would provide near maximum yield. A reference strip broadcast fertilized with ≈392 kg N ha^{-1} just before transplanting should be adequate for moderately to well–drained soils similar to those used in this study, which produce yields similar to many of the burley tobacco fields in central and western Kentucky (Atkinson and Sims, 1973).

An influence of environmental effects (location and year) on the relationship between NO_3^- concentration of air-cured lamina of burley tobacco and early-season plant NO_3^- concentration was absent, but separate functions are needed for leaves from top, middle, and bottom stalk positions (Fig. 5). Different equations for these three stalk positions is consistent with the wide range in NO_3^- concentrations that decreases markedly from the bottom to top leaves of mature burley tobacco (Bowman, 1972; Broaddus et al., 1965; Hamilton et al., 1982). The early-season plant NO_3^- test used in this study was a much better predictor of air-cured leaf NO_3^- than was early-season concentration of NO_3^- in the top 30 cm of soil (MacKown et al., 1999).

SUMMARY AND CONCLUSIONS

The use of plant NO_3^- concentration as an early-season diagnostic test to predict N sufficiency and NO_3^- concentration of cured leaf lamina offers promise for better N management of burley tobacco. Such a test during the interval between 3 and 5 wk after transplanting fits well into the normal cultivation practices for weed control, and it would offer the opportunity to correct deficiencies by banding N. Normally, the last cultivation takes place when stalk elongation is still limited at ≈5 wk after transplanting and before the rapid linear phase of tobacco growth and nutrient accumulation (Atkinson et al., 1977). With further research, the early-season plant dry matter NO_3^- test could be adapted readily to a field quick-test procedure, and the requirement for a N fertilizer strip that was not yield limiting is not unreasonable and could easily be incorporated into a nutrient management plan. However, before early-season N adjustment practices could be adopted, further research is required to properly calibrate the amount of additional N required to overcome the N deficit measured. Previously, we demonstrated that N fertilizer efficiency was enhanced and cured leaf yields were not adversely affected when 168 kg N ha^{-1} was banded ≈5 wk after transplanting instead of being broadcast just before transplanting (MacKown and Sutton, 1997; MacKown et al., 1999). However, the NO_3^- concentration of cured leaves from the bottom and middle stalk positions increased when the entire amount of N was banded at ≈5 wk after transplanting (MacKown et al., 1999). From a practical viewpoint, waiting until 5 wk after transplanting to apply the entire amount of N presents a yield risk, if weather were to delay or prevent banding N. Consequently, a portion of the N should be applied just before or after transplanting and the remainder during the first 5 wk after transplanting. This practice would probably offer the N fertilizer recovery benefit and lessen the NO_3^- concentration of cured leaves from the bottom and middle stalk positions when the entire amount of N is banded at ≈5 wk after transplanting, which would be desirable, if leaf buyers were to offer an economic incentive for cured leaf with low NO_3^-.

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